Comparison of leucine-rich repeat-containing G protein-coupled receptor 5 expression in different cancer and normal cell lines

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Abstract. Evaluating the expression of leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) may be useful for predicting the best models and achieving more accurate results in cancer research. Therefore, the aim of the present study was to analyze the LGR5 expression levels in different cell lines. Eight commonly used cell lines were assessed (COS-7, NIH3T3, HEK293, VERO, HeLa, BHK, HepG2 and AGS). All the cell lines were cultured in RPMI-1640 medium contain 10% fetal calf serum at 37°C in humidified conditions with 5% CO_2. According to the western blotting results, LGR5 was expressed in all cell lines. Densitometry results of LGR5 expression in the different cell lines showed that high LGR5 expression levels were apparent in BHK, AGS, VERO and NIH3T3 cell lines compared with the other cell lines. The results indicate that for the normal and cancer cell lines, BNK and AGS may be a better choice, respectively, for *in vitro* cancer studies.

Introduction

Cancer has become the major cause of mortality in certain countries in the 21st century (1). There are three models for cancer heterogeneity. Tumor heterogeneity is attained through genetic or epigenetic modifications. The stochastic model, as the first model, indicates that all tumor cells potentially are capable of self-renewal or differentiation, and are tumorigenic (2). In the second model, the hierarchical model, which is also known as the cancer stem cell (CSC) model of tumor growth, the ability of self-renew is considered for only a subset of tumor cells; the CSCs. These cells generate committed progenitor cells with limited proliferative potential, which ultimately lead to terminal differentiation (3-5). The third model is known as the complex model and this model suggests that epigenetic changes can potentially influence the tumor cell phenotype and function due to micro-environmental factors, thereby influencing tumor heterogeneity (2).

Recently, a new CSC theory, known as tumor stem cells or tumor-initiating cells, has emerged. A CSC was precisely defined by the American Association for Cancer Research in 2006 as a cell within a tumor that has positive susceptibility to self-renew and to reason the heterogeneous progeny of cancer cells that are contained within the tumor (6). The CSC model was previously described for hematological malignancies in 1997 (7).

Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is considered an intestinal stem cell marker (8). LGR5 has a transducer role in the Wnt signaling pathway (9,10). This signaling pathway is well known to be involved in the embryogenesis and carcinogenesis process (11). Also recognized as GPR49, LGR5 is a member of the G protein-coupled receptors, the largest family of cell-surface molecules involved in a signaling pathway. The size of the LGR5 gene is ~144 kb and it is located at position 12q22-q23 of chromosome 12. The LGR5 protein has seven transmembrane domains. Experimental findings showed that this protein in the mature form contains <17 leucine-rich repeats, each composed of 24 amino acids (2). The ligand for LGR5 is R-spondin, and following ligand-receptor binding, it forms a protein complex with frizzled lipoprotein receptor-related proteins 5 and 6. Subsequently, this complex positively regulates the Wnt signaling pathway (12,13).

Despite the controversies regarding the CSC model, CSC markers have the potential to provide a basis for new innovative targeted therapy for origins of cancer (14,15) and selecting the best cell line for LGR5-related studies. This may help in obtaining more accurate results. Therefore, the aim of the present study was to compare the LGR5 expression in different cancer and normal cell lines by western blot analysis.

Materials and methods

Cells and cell culture. Eight commonly used cell lines, including COS-7 (fibroblast-like kidney cells), NIH3T3 (mouse embryonic fibroblast cell line), HEK293 (human embryonic kidney cells), VERO (fibroblast-like kidney cell from African green monkey), HeLa (human epithelial carcinoma cell line),

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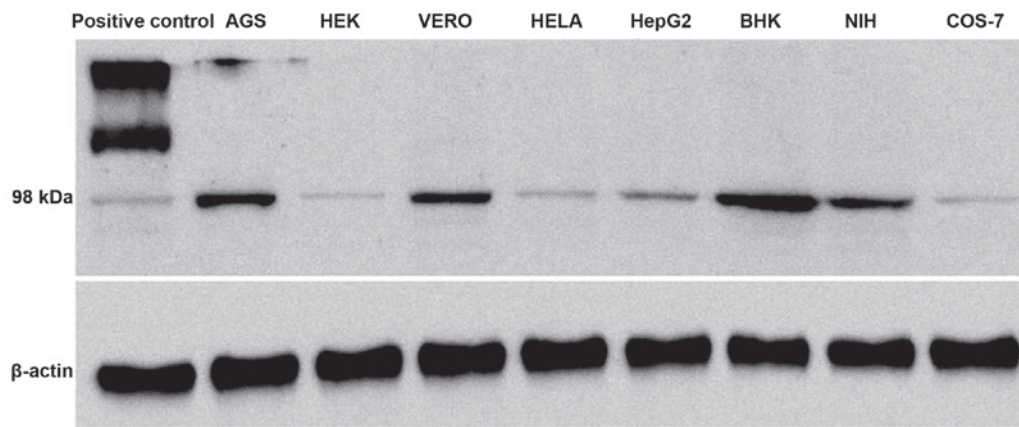E-mail: rafiei1710@gmail.com

Figure 1. Leucine-rich repeat-containing G protein-coupled receptor 5 protein expression levels in different cell linesin comparison with β-actin.

BHK (baby hamster kidney fibroblasts), HepG2 (human hepatocellular liver carcinoma cell line) and AGS (human gastric adenocarcinoma) were used in the study. All the cell lines were purchased from the National Cell Bank of the Iran Pasture Institute (Tehran, Iran). RPMI-1640 supplemented with 10% fetal calf serum, 100 IU/ml penicillin, and 10 μg/ml streptomycin (PAA Laboratories GmbH, Pasching, Austria) was utilized as the medium. The cells were cultured in humidified conditions at 37˚C and in 5% CO_2.

Following the exponential phase of growth, the cells were washed twice by ice-cold phosphate buffered saline (PBS), and adherent cells were scraped off from the flask by a cell scraper. Following this, all cells were resuspended in 1 ml of radioimmunoprecipitation assay buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), which included 2 mM phenylmethylsulfonyl fluoride, 10 μl of protease inhibitor cocktail and 1 mM sodium orthovanadate (Santa Cruz Biotechnology, Inc.). After centrifugation at 10,000 x g at 4˚C for 20 min, cell debris was removed and the supernatant was used for western blotting. The Bradford assay protocol was utilized in order to determine the protein concentrations (16).

Western blotting. Equal amounts of total protein from each cell line were separated on a 12.5% discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in a Mini-PROTEAN® Tetra Handcast System (Bio-Rad, Hercules, CA, USA) for 90 min at 120 V. Subsequently, the separated proteins were transferred to a polyvinylidene difluoride membrane (Santa Cruz Biotechnology, Inc.) in a tank-transfer system (Bio-Rad) at 100 V for 60 min in the presence of 0.1% SDS. Following this, the membrane was blocked with 5% skimmed dry milk in Tris-buffered saline (pH 7.6) with 0.1% Tween-20 for 1 h at room temperature. The membrane was incubated using LGR5 mouse monoclonal antibody clone 2A2 (OriGene Technologies, Rockville, MD, USA; cat. no. TA503316), which was used as a specific primary antibody (diluted to 1:2,000).

The blots were washed three times in PBS-Tween and goat anti-mouse IgG-HRP (Santa Cruz Biotechnology, Inc.; cat. no. SC-2005) secondary antibody was used for visualizing the antibody-antigen complex. The blots were developed with a Supersignal West Pico Chemiluminescent Substrate kit (Pierce, Rockford, IL, USA) for 5 min and images were captured by a Gbox device (Syngene, Cambridge, UK). To correct for protein loading and transfer efficiency, β-actin was used as the reference proteins for normalization in western blotting. By comparing with known protein size markers, the molecular weights were determined. Western blot band densities were quantitated by the GeneTools software (Syngene).

Results

Protein expression of LGR5 in the different cell lines. The expression pattern of the LGR5 levels in certain commonly used laboratory cell lines, which were AGS, HeLa, HEK293, HepG2, BHK, VERO, COS-7 and NIH3T3, was assessed. Western blotting on the total cell lysate was carried out to determine whether LGR5 was expressed at protein levels in these cells. All the cell lines tested showed a detectable amount of LGR5 expression; however, the level of expression differed in these cells. Expression of the LGR5 protein in the different cell lines in comparison with β-actin is shown in Fig. 1. A high level of LGR5 expression was detected in the AGS, BHK, VERO and NIH cell lines, while expression was barely detected in the other tested cell lines.

Relative expression of the LGR5 protein. To estimate the relative expression of LGR5 in the tested cell lines, the band density for each cell line was determined using densitometry (Table I). The results of the normalized band densities showed that, as expected, the ASG cells expressed a higher level of LGR5

Table I. Densitometry results of leucine-rich repeat-containing G protein-coupled receptor 5 expression in different cell lines.

Type of cell line	Cell line name	Densitometry result
Cancer	AGS	20.81
Normal	HEK-292	1.34
Normal	VERO	18.39
Cancer	HeLa	2.96
Cancer	HepG2	6.37
Normal	BHK	30.83
Normal	NIH3T3	17.20
Normal	COS-7	2.10

compared to the other tested cell lines. The BHK cells showed a higher level of LGR5 expression compared to the AGS cells. AGS cells are gastrointestinal cancer cells that are known to have an extremely high level of LGR5 expression, while BHK is an immortalized normal kidney cell of hamsters. Different amounts of LGR5 expression levels were also detected in two kidney-derived cells from monkey; VERO and COS-7 cells. VERO cells had a higher expression of LGR5 in comparison to COS-7 cells, which may reflect their distinct cell lineage in the kidney.

Discussion

CSCs have been a milestone in the investigations on cancer studies as they provide a noteworthy cellular mechanism to account for the therapeutic resistance and silent behavior exhibited by numerous tumors (17). LGR5 is an important target of the Wnt/β-catenin signaling pathway, which was first identified as an intestinal stem cells marker (10). The present study used western blotting to assess the expression levels of LGR5 in eight commonly used laboratory cell lines, which were AGS, HEK, VERO, HeLa, HepG2, BHK, NIH3T3 and COS-7. The results showed that the expression of the LGR5 protein in the BHK and AGS cell lines were higher compared to the other cells. The HEK-292 and COS-7 cell lines expressed lower levels of LGR5 compared with other cells. Furthermore, the LGR5 expression in cancer cell lines was higher compared to the normal cells.

He *et al* (18) demonstrated various levels of LGR5 expression in five colorectal cancer cell lines by quantitative RT-PCR. The study reported high LGR5 expression levels in SW620, Caco-2 and SW480 cells, and low levels in LoVo and HCT116 (18). Another study carried out by Ku *et al* (19) focused on the establishment of 13 human colorectal carcinoma cell lines. The CSC biomarker cluster of differentiation 133 (CD133) was expressed in 12 of the cell lines, while the biomarkers CD44 and LGR5 were expressed in all 13 cell lines (19). In conclusion, the present findings suggest that the expression levels of LGR5 varied in different cell lines, and there were high expression levels of LGR5 in BHK and AGS for the normal and cancer cell lines, respectively. Therefore, these two cells lines are the best options for *in vitro* cancer studies.

Acknowledgements

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